**Lamina-Mediated Regulation of the DNA Damage Response and Chromatin Architecture at Site-Specific Double-Strand Breaks**

**Project Summary**

In eukaryotic cells, the DNA damage response (DDR) operates within a highly dynamic chromatin environment, integrating DNA repair with transcription, replication, chromatin remodeling, and three-dimensional genome organization. The nuclear lamina—a meshwork of intermediate filaments composed of A- and B-type lamins—serves not only as a structural scaffold maintaining nuclear architecture but also as a platform for chromatin interaction and genome regulation. Emerging evidence suggests a functional role for the nuclear lamina in the DDR and therapy-induced genomic remodeling in cancer.

To investigate the contribution of lamin A/C to double-strand break (DSB) repair and chromatin reorganization, we established an MCF10A epithelial cell model expressing an inducible AsiSI restriction enzyme. Treatment with 4-hydroxytamoxifen (4-OHT) activates AsiSI, generating ~150 spatially defined DSBs across the genome. Using CRISPR/Cas9, we generated **LMNA knockout (LMNA-KO) AsiSI-ER** cell lines to compare DDR processes in lamin A/C-deficient versus control contexts.

Initial data show that **LMNA-deficient cells display defective formation of γH2AX clusters**, implicating the nuclear lamina in DSB-induced chromatin remodeling and efficient DDR signaling. To dissect this process, we will employ **ChIP-seq** for repair factors and histone modifications, and **Hi-C** to capture DSB-induced changes in chromatin architecture. Quantitative imaging will be used to assess γH2AX foci dynamics and spatial organization in real time.

**Project Goals**

* To determine how lamin A/C influences the recruitment of DNA repair machinery to site-specific DSBs
* To define changes in 3D chromatin conformation upon DSB induction in LMNA-KO versus control cells

**Supervision and Lab Environment**

This research is conducted under the supervision of **Prof. Barbara Majello**, an expert in epigenetics and chromatin biology. The project is based in the **Department of Biology, University of Naples Federico II**. The **Majello Lab** (<http://www.majellolab.unina.it/>) offers access to advanced molecular biology techniques, next-generation sequencing platforms, high-throughput imaging systems, and bioinformatics support.

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